

# PATENT SPECIFICATION

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## COMPLETE SPECIFICATION

### Method of preparing Pertussis Toxin and Toxoid

We, **LEDERLE LABORATORIES, INC.**, a corporation organized and existing under the laws of the State of Delaware, United States of America, of 30, Rockefeller Plaza, City and State of New York, United States of America, do hereby declare the nature of this invention and in what manner the same is to be performed, to be particularly described and ascertained in and by the following statement:—

This invention relates to pertussis toxin and toxoid and to methods of preparing the same.

The subject of immunization against whooping cough and the treatment of this disease has been given extensive study. The results obtained heretofore have not been especially effective and the need for suitable materials for immunization against whooping cough is obvious in view of the prevalence of this ailment.

According to the present invention these difficulties are overcome by a method in which cultures of pertussis bacilli are grown in a medium of soluble-starch broth, vegetable extract broth or semi-solid agar, whereupon the bacteria are separated from the medium by filtration and the filtrate is preserved for use as a toxin, or, if desired, it is detoxified to form a toxoid.

#### EXAMPLE:

Cultures of pertussis bacilli are grown in shallow layers (about  $\frac{1}{2}$  inch) of soluble-starch broth in an atmosphere containing about 20% carbon dioxide and 80% oxygen. This growing or incubation period extends over about 48 hours at a temperature of about 37° C. At the end of this period of time, the bacteria are separated from the broth by a filtration operation through paper and then through a Berkefeld or Mandler filter to give a bacteria-free filtrate, the bacteria remaining on the filters. The filtrate constitutes the toxin and if it is to be used as such, it is suitably preserved by the addition of phenol, a mercurial preservative, or some similar material.

If the toxoid is desired, it is prepared by adding about 0.3% (by volume) formalin (40%) to the unpreserved toxin,

prepared as described above, and incubating this mixture at a temperature of about 40° C. for 2 to 4 weeks. This toxoid is then preserved by the addition of a preservative material as is described for the toxin.

The pertussis toxin prepared as described may be used as a skin test for determining susceptibility to whooping cough. The pertussis toxoid may be used for immunization against or for the treatment of whooping cough.

In place of using soluble starch, the bacilli may be grown in any suitable vegetable extract broth, the potato extract being especially effective for this purpose. However, it is important that the broth be used in shallow layers, say not over 1 inch. Another medium for incubation of the bacilli is semi-solid agar and this can be used in thicker layers, say about 2 inches.

The atmosphere in which the incubation takes place is preferably one containing a mixture of carbon dioxide and oxygen in substantial quantities although the proportions thereof are not necessarily limited to those given in the specific example. As a matter of fact the incubation may be carried out in ordinary air if desired.

The time and temperature for incubation are also subject to some variation although the 48 hour period is approximately the minimum for commercial production at the temperature utilized. Any considerable extension of this period may cause undesirable side reactions.

In preparing the toxoid the amount of the formalin which may be used may be changed from that given although it is obviously undesirable to use more formalin than is required to detoxify the toxin. The time and temperature used for the detoxification is also subject to some variation.

Our toxin and toxoid have proved themselves effective in clinical and bacteriological tests on rabbits and humans. When our toxin is injected intradermally into rabbits, there is produced a swelling and erythema in dilutions up to 1—100 and in most cases, the undiluted toxin

causes necrosis somewhat similar to that produced by the injection of whole cultures. The toxoid produced by our detoxification treatment, upon injection  
5 into rabbits, produces antibodies which neutralize the toxin, as evidenced by injections of such toxin. Our toxoid also neutralizes the necrotic factor of whole cultures as evidenced by the lack of  
10 necrosis when live cultures are injected into rabbits previously immunized with the toxoid. It is evident that our toxoid injections produce something akin to a true antitoxin with its attendant  
15 immunity.

In a series of clinical studies under the direction of competent pediatricians, our pertussis toxoid has been found especially effective for the early treatment of active  
20 cases of whooping cough. It has also been found effective as a prophylactic in cases that have been directly exposed to the disease. If used early in treatment or soon after exposure in contact cases, the immunological response to our toxoid is  
25 sufficiently rapid to prevent or modify the disease in the large majority of cases.

In one series of tests a confirmed clinical and laboratory diagnosis of  
30 whooping cough was made on a total of 160 treated cases, divided into three groups. The first group containing 53 cases were treated during the first or second week after onset and before  
35 paroxysms had developed. In the second group, 70 cases were treated during the third or fourth week of the infection in which there was already some degree of whooping and vomiting. In the third  
40 group there were 37 cases in which treatment was begun during the fifth week of the disease. Of the first group treated 92% of the cases did not develop whooping or vomiting and of these who did not  
45 develop severe symptoms 61% cleared up entirely after the fourth injection (each dose 1.5 cc. every other day). In several instances there was complete cessation of symptoms within 2 or 3 days when treatment was begun in the early stages and  
50 while this may be due to an unusually prompt increase in antibody such as may occur when a partially immunized person is injected with antigen, it also suggests  
55 the possibility that our pertussis toxoid produces a desensitizing effect which may be responsible, in some measure, for its curative effect.

In the second group, all symptoms  
60 disappeared after the fourth injection in 43% of the individuals treated, so that the diagnosis, by another observer, was extremely difficult. In the remainder of this group the results were varied but in  
65 each case there was marked improvement,

the disease continuing to run a mild, modified course.

Those cases that were treated during the fifth week of the disease were refractory to treatment.

In none of the treated cases was there any evidence of an interstitial or a broncho-pneumonia. Obviously the earlier the treatment is instituted for exposed cases or in children suffering from the  
75 infection, the greater the opportunity to effect an immunity in time to prevent the disease or to modify its course. Among 140 children not directly exposed, who had received the complete prophylactic treatment, 3 developed mild and a typical whooping cough within three weeks after the last injection. Two of these 3 cases occurred in families where non-immunized children suffered typical and severe  
85 whooping cough. Of 10 intimate contacts who received our pertussis toxoid immediately after exposure, all with the exception of 2 escaped the disease and even these 2 were mild in character.

Naturally the dosage may be varied to meet conditions. The following table indicates the average dosage but, of course, may be varied if desired:

#### DOSAGE.

For curative use: 3 to 5 injections, 2 cc. every other day.

For treatment of exposed contacts: 3 injections, 2 cc. every other day.

For general prophylactic use: (children not directly exposed), 3 injections, 2 cc. each at weekly intervals.

On the whole, the reactions from the injections have been extremely mild or almost completely lacking. Where there is any objectionable reaction, it responds promptly to suitable treatment and it is possible to continue injection using an increased number of doses of smaller amounts of the toxoid.

It is sometimes desirable to prepare a vaccine-toxoid mixture to be used to give anti-bacterial immunity as well as the anti-toxic immunity. Such a mixture can be prepared by taking the broth after  
115 incubation and detoxifying it without filtration, using formalin for this purpose, as already described. This treatment detoxifies the bacteria as well as the toxin. Additional vaccine, independently prepared, may be added, if desired. Still another way in which the mixture can be made up is to mix toxoid and vaccine, each prepared separately.

Having now particularly described and  
125 ascertained the nature of our said invention and in what manner the same is to be performed, we declare that what we claim is:—

1. A process which comprises growing 130

cultures of pertussis bacilli in a medium of soluble-starch broth, vegetable extract broth or semi-solid agar, thereafter separating the bacteria from the medium  
5 by filtration and preserving the filtrate for use as a toxin or, if desired, detoxifying it to form a toxoid.

2. A process according to Claim 1 in which the pertussis bacilli is cultivated  
10 for a period of about 48 hours at a temperature of 37° C. and under an atmosphere containing about 1 part by volume of carbon dioxide and about 4 parts by volume of oxygen.

15 3. A process according to Claim 1 or 2 in which the filtrate is detoxified by

adding a small amount of formalin thereto and by maintaining it at a suitable temperature to convert the toxin contained in the liquid to a toxoid. 20

4. A process according to Claim 3 in which about 0.3% by volume of formalin is added and the mixture is incubated at a temperature of about 40° C. for from 2 to 4 weeks. 25

5. The process substantially as hereinbefore described.

6. Pertussis toxin or toxoid whenever prepared by the process hereinbefore particularly described and ascertained.

Dated this 12th day of August, 1938.

MARKS & CLERK.